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MARK-AGE population: From the human model to new insights

Miriam Capri[a,b,*,1], María Moreno-Villanueva[c,1], Elisa Cevenini[a], Elisa Pini[a], Maria Scurti[a], Vincenzo Borelli[a], Maria Giustina Palmas[b], Marco Zoli[d], Christiane Schön[e], Anne Siepelmeyer[e], Jürgen Bernhardt[e], Simone Fiegl[f], Gerben Zondag[g], Anton J.M. de Craen[h], Antti Hervonen[i], Mikko Hurme[i], Ewa Sikora[j], Efstathios S. Gonos[k], Konstantinos Voutetakis[k], Olivier Toussaint[l], Florence Debacq-Chainiaux[l], Beatrix Grubeck-Loebenstein[m], Alexander Bürkle[c], Claudio Franceschi[a,b]

[a] DIMES-Department of Experimental, Diagnostic and Specialty Medicine, Alma Mater Studiorum, University of Bologna, 40126 Bologna, Italy
[b] CIG-Interdepartmental Centre "L.Galvani", Alma Mater Studiorum, University of Bologna, 40126 Bologna, Italy
[c] Molecular Toxicology Group, Department of Biology, University of Konstanz, 78457 Konstanz, Germany
[d] Department of Medical and Surgical Sciences, University of Bologna and S. Orsola-Malpighi Hospital, Bologna, Italy
[e] BioTeSys GmbH, 73728 Esslingen, Germany
[f] UMIT Private Universität für Gesundheitswissenschaften, Medizinische Informatik und Technik GmbH, 6060 Hall in Tirol, Austria
[g] DNage BV, Leiden, The Netherlands
[h] Department of Gerontology and Geriatrics, Leiden University Medical Centre, Leiden, The Netherlands
[i] School of Medicine, University of Tampere, 33014 Tampere, Finland
[j] Laboratory of the Molecular Bases of Ageing, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland
[k] Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, 48 Vas. Constantinou Ave. 116 35 Athens, Greece
[l] NARILIS URBC, University of Namur (FUNDP), 61, rue de Bruxelles, 5000 Namur, Belgium
[m] Immunology Division, Institute for Biomedical Aging Research, Universität Innsbruck, Rennweg 10, 6020 Innsbruck, Austria

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Many relevant EU projects were funded during last decade and MARK-AGE (European Study to Establish Biomarkers of Human Ageing) was the first focused on the identification of biomarkers connecting itself with a previous funded project, i.e. GEHA (Genetic of Healthy Ageing) that identified European families with longevity component (Franceschi et al., 2007). MARK-AGE was mainly a cross sectional study, based on definite assumptions, as it will be described below, and focused on an age range between 34 and 75 years, in order to identify early biomarkers of biological vs. chronological age, potentially capable of predicting the rate of ageing later in life. Complementarily, many anti-ageing strategies have been proposed, such as those related to immune system remodelling (Capri et al., 2006b), but a new era begun on different tissues-specific epigenomics (Horvath, 2013; Romanoski et al., 2015), food/nutrition science, diet intervention (Santoro et al., 2014; Bacalini et al., 2014; Mercken et al., 2013; Cevenini et al., 2013; Berendsen et al., 2014) and its interaction with genomic background (Corella and Ordovás, 2014) and gut microbiota remodeling (Ottaviani et al., 2011; Biagi et al., 2013; Collino et al., 2013). These recent findings together with MARKAGE biomarkers not only give a new perspective in term of ageing rate measurement, but also new insights for molecular-targeted interventions to slow down human ageing process and likely, age-related pathologies onset.

Abbreviations: CS, Cockayne syndrome; DS, Down syndrome; GO, GEHA offspring; RASIG, randomly recruited age-stratified individuals from the general population; SGO, spouses of GO; WS, Werner syndrome.

* Corresponding author at: Department of Experimental, Diagnostic and Specialty Medicine, Alma Mater Studiorum, University of Bologna, Via S. Giacomo, 12 40126 Bologna, Italy.
E-mail address: miriam.capri@unibo.it (M. Capri).
[1] These authors contributed equally to this work

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1. Ageing of European Countries

The proportion of elderly people over 65 years in Europe (EU-28Countries) is predicted to increase from 18.2% in 2013 to 28.7% by 2080 (Eurostat source). The share of those aged 80 years or above in the EU-28's population is projected to almost triple between 2013 and 2080. Thus, over the coming decades, Europe's demographic makeup will change harshly. Our populations are becoming older than ever before due to three major trends. First, the baby-boom generation approaches retirement age thus older people will rise rapidly; second, birth rates have remained low for several decades; and third, European people, but not only, are living longer lives, even if comorbidites and disabilities are increasing, especially after 62 years (Eurostat source). In 2011 the number of healthy life years at birth was estimated at around 62 years for men and 61 years for women in EU-28, meaning that approximately, 16% and 21% of unhealthy life for men and women, respectively, are expected. This demographic remodelling emphasises the critical importance of identifying new strategies able to counteract or slow down ageing and the onset of age-related diseases and disabilities. These new strategies can contribute to increase the number of elderly citizens in good health, and reducing age-related medical and social costs. Therefore the identification of biomarkers of healthy or unhealthy ageing (see Bürkle et al., this issue) and the adoption of healthy life styles before the use of drug-based therapies are necessary. This situation has pushed EU governance to identify scientific projects able to find solutions to monitor and counteract unhealthy ageing, thus promoting a healthy and active ageing.

2. From population to individual level

On the other side, the rate of ageing in humans is not uniform due to the complexity of genetics and epigenetics (Capri et al., 2006a, 2014), which interact with environment (Biagi et al., 2013; Garm et al., 2013) and stochasticity with diverse weights at different phases of life taking into account also embryonic and foetal development (Cevenini et al., 2010). Further, these main factors could differently affect the rate of ageing at the levels of cells, tissues or body systems within the same organism according to the hypothesis of the "mosaic of ageing" (Cevenini et al., 2008). Tissues and organs might age at different rate and furthermore, individuals of same chronological age might have different biological age. This complexity makes more difficult the identification of a unique comprehensive mechanism of ageing and related biomarkers (Deelen et al., 2013).

3. The human model in MARK-AGE: innovative concepts

MARK-AGE aimed at the identification of biomarkers of ageing capable of distinguishing between chronological and biological ageing (see Giampieri et al., this issue) looking at systemic parameters assessed in the blood/urine and buccal mucosa cells (BMC) of volunteers, likely mirroring the entire organism. To achieve this objective a robust human model with definite assumptions was conceptualised accordingly (see Bürkle et al., this issue), as briefly described:

1. Subjects representing the "normal" aging: randomly recruited age-stratified individuals from the general population or RASIG, covering the age range 35–74 years;
2. Subjects representing the successful or "decelerate" aging: subjects born from a long-living parent belonging to a family with long living sibling(s) already recruited in the framework of the GEHA -Genetic of Healthy Ageing- project (Skytthe et al., 2011). These individuals ("GEHA offspring" or GO) were recruited together with their spouses or SGO representing the best control to evaluate possible life style effects, since they have shared the same environmental factors for many years with their partners.
3. Subjects representing accelerated aging: patients with progeroid syndromes (Cockayne, Werner and Down syndromes), characterised by accelerated "segmental" ageing.

4. Independent variables in MARK-AGE

MARK-AGE model took into account essential independent variables, i.e. gender, age, geography and populations. The selection of these variables was done on the basis of specific assumptions such as the major role of gender in the ageing process, the need to identify biomarkers before the late phase of life and the role of environment/geography/culture in the population ageing with a consistent number of individuals.). About 5% of recruited volunteers were not classified as "successfully recruited" MARK-AGE individual, either because exclusion criteria such as positive results to HBV, HCV viruses screening or due to errors in data entry into the phenotypic database. The

a GENDER. Males and females were recruited close to 50% in each age class, giving the possibility to analyse age-gender effects. Literature strongly suggests a gender effect on mortality rate. Females live longer even with a higher frequency of disabilities and comorbidities (Oksuzyan et al., 2008). This difference between males and females seems to be a paradox and the biological causes are not yet understood, but many data suggest the involvement of sexual chromosomes and their age-dependent methylation pattern (Capri et al., 2014; Gentilini et al., 2012, 2013). Furthermore, prospect demographic studies show that the gap of life expectancy between genders will become smaller in the next 40 years, even if an analysis comparing healthy life years between the gender at the age of 65 shows there were 9 EU Member States in 2011 where men could expect more health life years than women (Eurostat source).
b AGE. An age range between 34 and 75 years was thought to be crucial to identify early biomarkers of ageing. Humans undergo different phases of life and 15 years beyond 50–60 years, i.e. the transition between adult age and the beginning of the consistent ageing process appear to be a critical temporal window. Indeed,

Table 1
Individuals classified as "successfully recruited" RASIG, GO, SGO and DS volunteers finally inserted in the database and considered for MARK-AGE analysis.

Centres	RASIGM	RASIGF	GOM	GOF	SGOM	SGOF	DSM	DSF	MissedCodes	Sub total
BioTeSys	162	188							8	358
FUNDP	121	135	33	42	17	21			8	377
LUMC			52	68	58	44			0	222
NENCKI	192	189	25	51	30	17			2	506
NHRF	200	203	5	17	4	2			1	432
UIBK	200	190							0	390
UNIBO	193	199	52	42	22	33	27	20	0	588
UTA	27	63	53	88	24	33			8	296
Total	1095	1167	220	308	155	150	27	20	27	3169

Table 2
Strategies of MARKAGE dissemination applied by all the centres involved in the recruitment.

Centres	Population	Dissemination and strategy of recruitment
BioTeSys GmbH (DE)	RASIG	Newspaper articles, information evening at the town hall together with the governing mayor; registration office (letter/flyer), volunteers known from other trials conducted at BioTeSys a little word-of-mouth recommendation.
FUNDP/StratiCELL (BE)	RASIG/GO/SGO	Contacted an open-university for persons belonging to the 3rd age and all societal horizons; the Services of the Human Resources of the City of Namur, the Univ. of Namur, Univ. Clinics of Mont-Godinne, and Regional Hospital Centre, dealing with all sorts of personnel; organised press conference at Univ. of Namur (many press articles, interviews on national radios, local TV news, national TV programme on ageing). GEHA reference for GO list
LUMC (NL)	GO/SGO	Drafted a list with a number of picked nominatives (GEHA reference).
NHRF (GR)	RASIG/GO/SGO	Contact by email all the personnel of Research Institutes of Athens. i. Contact by phone, call all the GEHA siblings giving information to GO/SGO on MARK-AGE, sending them the informative sheet by post or fax. ii. Contact by phone, call all our personal acquaintances giving information on MARK-AGE
NENCKI (PL)	RASIG/GO/SGO	Obtained addresses of 3200 RASIG from Ministry of Interior and Administration (presently Ministry of Interior) according to PESEL (National Electronic Census Number System); sent 1700 letters of invitation, the responders feedback by phone or e-mail (22%). GEHA reference for GO list.
UIBK (AT)	RASIG	Articles in a very common Tyrolean daily newspaper; dissemination on local TV news and articles in other newspapers and magazines.
UNIBO (IT)	RASIG/GO/SGO/DS	GO/SGO were recruited before RASIG: drafted a list with a number of picked nominatives. RASIG: population of PIANORO (17,000 inhabitants) near Bologna; contacts with the Mayor Citizen and the District of Public Health. A presentation of the project was performed to all the population with local Government and general practitioners; local paper, information flyers; magazine associated to food discounts) and TV, recruited also persons from the University of Bologna. DS and their family were contacted by specific association in Bologna
UTA (FL)	RASIG/GO/SGO	Newspapers, local TV announcements

this period includes the menopause in women and the period where major age related diseases and the rate of mortality start to increase (Rauser et al., 2006). Accordingly, one of the major characteristic of MARK-AGE is to focus on the above mentioned age range in order to identify early biomarkers of biological vs. chronological age, potentially capable of predicting the rate of ageing later in life.

c GEOGRAPHY. 11 different European beneficiaries, distributed from North to South, were responsible for the recruitment. This distribution has given the possibility to study environmental/geographical/cultural population effects. Thus, geographical or environmental factors could influence parameters, such as methylation patterns (Pirazzini et al., 2012), but differences among diverse latitudes can be revealed and ad hoc analysed.

d POPULATIONS. One of the main goal of MARK-AGE was to recruit consistent groups of subjects assumed to have diverse rates of ageing, as described above. On the whole 3337 subjects were initially recruited and the recruitment of GO/SGO was the most challenging due to large distances between subjects residence and laboratory place, thus it was more time consuming, more expensive and more effort needed to fulfil requirements for transport of biological samples. Furthermore, the number of SGO was about half of GO since somebody declined, divorced or passed away. 53 Down Syndrome individuals were recruited by UNIBO with a specific strategy committed to family involvement and the employment of specialized human resources, such as psychologists and psychiatrists. Further a specific battery of tests focused on behaviour, neuropsychological, neuropsychiatric and cognitive assessment was set up (see Moreno-Villanueva, Capri et al., this issue) in order to evaluate the functional and cognitive status of these people. DNage B.V. and later on University of Constance (UKON, Germany) were responsible for collection of about 59 existing blood samples from WS patients and a minor number of CS patients recruited by external collaborators. In view of the complexity and the size of the MARK-AGE project, dropouts were expected. Nevertheless, about 95% of recruited subjects (i.e. 3169) could be included in the MARK-AGE population for analysis (Table 1 final numbers of successfully recruited volunteers and also inserted in the MARK-AGE database were the following: 2262 RASIG; 528 GO; 305 SGO and 47 DS as shown in Table 1 (59 WS patients samples are not included). Technical aspects are briefly reported in Box I and Table 2. All procedures of data base management and cleaning are described (see Baur et al., this issue).

Box I: Practical aspects of MARK-AGE.

- MARK-AGE RASIG population was recruited by BioTeSys GmbH (Germany), University of Namur (FUNDP, Belgium), Österreichische Akademie der Wissenschaften (UIBK, Austria), Institute of Experimental Biology (NENCKI, Poland), National Hellenic Research Foundation Centre (NHRF, Greece), University of Tampere (UTA, Finland) and University of Bologna ALMA MATER STUDIORUM (UNIBO, Italy). Subjects from GO/SGO cohorts were recruited by University of Leiden (LUMC, Netherland), University of Tampere (UTA, Finland), NENCKI, UNIBO, FUNDP and NHRF. UNIBO additionally recruited DS patients, DNage B.V. (Netherland) and University of Constance (UKON, Germany) were responsible for collection of existing blood samples from CS and WS patients recruited by external collaborators. A specific questionnaire was developed for RASIG/GO/SGO population, which was adapted by UNIBO to the DS patients. All the questionnaires and biological samples have been described in the article dedicated to MARK-AGE standard operating procedures (see Moreno-Villanueva, Capri et al., this issue). Recruitment strategies are detailed in Table 1. Reasonably, GO/SGO recruitment was carried out by Beneficiaries who were involved in GEHA project since they had already contacted these families before (Franceschi et al., 2007).
- Inclusion and exclusion criteria RASIG were randomly recruited age-stratified individuals from the general population (both sexes) aged 35–74 and able to give informed consent. Concerning GO subjects, they were sons or daughters of GEHA individuals and SGO were GO spouses in age-range 55–74 years. As well as RASIG, all of them should be able to give their informed consent. Exclusion criteria were the following: i. self-reported seropositivity for HIV, HBV (except seropositivity by vaccination) and HCV (HBV and HCV seropositivity assessed after blood collection). ii. presence of actual cancer and current use of anti-cancer drugs or glucocorticoids; iii. less than 50% of lifetime spent in country of residence. iv. inability to give informed consent.

5. Robust data, longitudinal component and progress

MARK-AGE was a great opportunity of advancement in knowledge. To ascertain the biological and analytical robustness of the measurements of candidate biomarkers, 100 donors from the whole study population were re-sampled within 3–6 months. Further, within the lifetime of the project (66 months), a limited (12%) random sample of probands were re-tested in order to establish

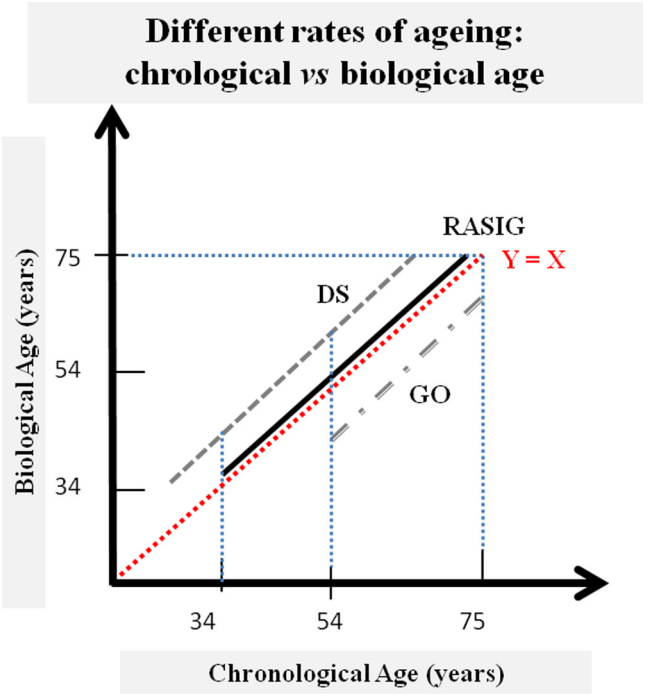

Fig. 1. Chronological vs biological age.
Distances from bisector ($Y = X$) are only indicative. Basic assumption of MARK-AGE highlights the possibility to identify systemic biomarkers which correlate with age and significantly separate RASIG, GO and DS populations of different age rates. RASIG: Randomly recruited Age-Stratified Individuals from the general population (age range: 34–75 years); GO: GEHA offspring (age range: 54–75 years); DS: Down syndrome (age range: 19–68 years)

a longitudinal component within the study. To make strongest the results emerging from MARK-AGE it would be important to perform a more robust longitudinal study on all the recruited subjects. Nevertheless, those parameters, which correlate with age and those, which significantly separate cohorts of different age rates are those able to monitor the systemic age-related changes of the body. Fig. 1 depicts the concepts which MARK-AGE faced and the basic mathematical approach from which algorithms have been developed.

The strongest MARK-AGE biomarkers and those related to the epigenetic clock (353CpG sites shaping an ageing clock in terms of chromatin states and tissue variance), recently raised up from studies on differently aged human tissues, human obesity and Down Syndrome (Horvath, 2013; Horvath et al., 2014, 2015), reveal powerful tissue -specific biomarkers mirroring the systemic condition. These findings open new questions on the future role of the biomarkers network which supports information of healthy status and likely could also "transport communication" among different cell, organs or tissues. Thus, new insights are expected by biomarkers network, not only highlighting tissue-specific and/or systemic molecular pathways, but also monitoring the tissue-specific and/or systemic rate of ageing, both at individual and population levels, and eventually targeting genes or their products for age-related pathology interventions.

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References

Bacalini, M.G., Friso, S., Olivieri, F., Pirazzini, C., Giuliani, C., Capri, M., Santoro, A., Franceschi, C., Garagnani, P., 2014. Present and future of anti-ageing epigenetic diets. Mech. Ageing Dev. 136–137, 101–115.

Berendsen, A., Santoro, A., Pini, E., Cevenini, E., Ostan, R., Pietruszka, B., Rolf, K., Cano, N., Caille, A., Lyon-Belgy, N., Fairweather, S., Feskens, E., Franceschi, C., 2014. A parallel randomised trial on the effect of a healthful diet on inflammageing and its consequences in European elderly people: design of the NU-AGE dietary intervention study. Mech. Ageing Dev. 136, 14–21.

Biagi, E., Candela, M., Turroni, S., Garagnani, P., Franceschi, C., Brigidi, P., 2013. Ageing and gut microbes: perspectives for health maintenance and longevity. Pharmacol. Res. 69, 11–20.

Capri, M., Salvioli, S., Sevini, F., Valensin, S., Celani, L., Monti, D., Pawelec, G., De Benedictis, G., Gonos, E.S., Franceschi, C., 2006a. The genetics of human longevity. Ann. N. Y. Acad. Sci. 1067, 252–263.

Capri, M., Monti, D., Salvioli, S., Lescai, F., Pierini, M., Altilia, S., Sevini, F., Valensin, S., Ostan, R., Bucci, L., Franceschi, C., 2006b. Complexity of anti-immunosenescence strategies in humans. Artif. Organs, 30730–30742.

Capri, M., Santoro, A., Garagnani, P., Bacalini, M.G., Pirazzini, C., Olivieri, F., Procopio, A.D., Salvioli, S., Franceschi, C., 2014. Genes of longevity: an endless quest? Curr. Vasc. Pharmacol. 12, 707–717.

Cevenini, E., Bellavista, E., Tieri, P., Castellani, G., Lescai, F., Francesconi, M., Mishto, M., Santoro, A., Valensin, S., Salvioli, S., Capri, M., Zaikin, A., Monti, D., de Magalhães, J.P., Franceschi, C., 2010. Systems biology and longevity: an emerging approach to identify innovative anti-aging targets and strategies. Curr. Pharm. Des. 16, 802–813.

Cevenini, E., Invidia, L., Lescai, F., Salvioli, S., Tieri, P., Castellani, G., Franceschi, C., 2008. Human models of aging and longevity. Expert Opin. Biol. Ther. 8, 1393–1405.

Cevenini, E., Monti, D., Franceschi, C., 2013. Inflamm-ageing. Curr. Opin. Clin. Nutr. Metab. Care 16, 14–20.

Corella, D., Ordovás, J.M., 2014. Aging and cardiovascular diseases: the role of gene-diet interactions. Ageing Res. Rev. 18, 53–73.

Collino, S., Montoliu, I., Martin, F.P., Scherer, M., Mari, D., Salvioli, S., Bucci, L., Ostan, R., Monti, D., Biagi, E., Brigidi, P., Franceschi, C., Rezzi, S., 2013. Metabolic signatures of extreme longevity in northern Italian centenarians reveal a complex remodeling of lipids, amino acids, and gut microbiota metabolism. PLoS One 8 (3), e56564.

Deelen, J., Beekman, M., Capri, M., Franceschi, C., Slagboom, P.E., 2013. Identifying the genomic determinants of aging and longevity in human population studies: progress and challenges. Bioessays 35, 386–396.

Eurostat. European Commission Statistics web site: http://ec.europa.eu/eurostat/statistics-explained/index.php/Healthy_life_years_statistics

Franceschi, C., Bezrukov, V., Blanché, H., Bolund, L., Christensen, K., de Benedictis, G., Deiana, L., Gonos, E., Hervonen, A., Yang, H., Jeune, B., Kirkwood, T.B., Kristensen, P., Leon, A., Pelicci, P.G., Peltonen, L., Poulain, M., Rea, I.M., Remacle, J., Robine, J.M., Schreiber, S., Sikora, E., Slagboom, P.E., Spazzafumo, L., Stazi, M.A., Toussaint, O., Vaupel, J.W., 2007. Genetics of healthy aging in Europe: the EU-integrated project GEHA (GEnetics of Healthy Aging). Ann. N. Y. Acad. Sci. 1100, 21–45.

Garm, C., Moreno-Villanueva, M., Bürkle, A., Larsen, L.A., Bohr, V.A., Christensen, K., Stevnsner, T., 2013. Genetic and environmental influence on DNA strand break repair: a twin study. Environ. Mol. Mutagen. 54, 414–420.

Gentilini, D., Castaldi, D., Mari, D., Monti, D., Franceschi, C., Di Blasio, A.M., Vitale, G., 2012. Age-dependent skewing of X chromosome inactivation appears delayed in centenarians' offspring. Is there a role for allelic imbalance in healthy aging and longevity? Aging Cell 11, 277–283.

Gentilini, D., Mari, D., Castaldi, D., Remondini, D., Ogliari, G., Ostan, R., Bucci, L., Sirchia, S.M., Tabano, S., Cavagnini, F., Monti, D., Franceschi, C., Di Blasio, A.M., Vitale, G., 2013. Role of epigenetics in human aging and longevity:

genome-wide DNA methylation profile in centenarians and centenarians' offspring. Age (Dordr) 35, 1961–1973.
Horvath, S., 2013. DNA methylation age of human tissues and cell types. Genome Biol. 14, R115.
Horvath, S., Erhart, W., Brosch, M., Ammerpohl, O., von Schönfels, W., Ahrens, M., Heits, N., Bell, J.T., Tsai, P.C., Spector, T.D., Deloukas, P., Siebert, R., Sipos, B., Becker, T., Röcken, C., Schafmayer, C., Hampe, J., 2014. Obesity accelerates epigenetic aging of human liver. Proc. Natl. Acad. Sci. U. S. A. 111, 15538–15543.
Horvath, S., Garagnani, P., Bacalini, M.G., Pirazzini, C., Salvioli, S., Gentilini, D., Di Blasio, A.M., Giuliani, C., Tung, S., Vinters, H.V., Franceschi, C., 2015. Accelerated epigenetic aging in down syndrome. Aging Cell 9 (Feburary), http://dx.doi.org/10.1111/acel.12325 [Epub ahead of print].
Mercken, E.M., Crosby, S.D., Lamming, D.W., JeBailey, L., Krzysik-Walker, S., Villareal, D.T., Capri, M., Franceschi, C., Zhang, Y., Becker, K., Sabatini, D.M., de Cabo, R., Fontana, L., 2013. Calorie restriction in humans inhibits the PI3K/AKT pathway and induces a younger transcription profile. Aging Cell 12, 645–651.
Oksuzyan, A., Juel, K., Vaupel, J.W., Christensen, K., 2008. Men: good health and high mortality: sex differences in health and aging. Aging Clin. Exp. Res. 20, 91–102.
Ottaviani, E., Ventura, N., Mandrioli, M., Candela, M., Franchini, A., Franceschi, C., 2011. Gut microbiota as a candidate for lifespan extension: an ecological/evolutionary perspective targeted on living organisms as metaorganisms. Biogerontology 12, 599–609.
Pirazzini, C., Giuliani, C., Bacalini, M.G., Boattini, A., Capri, M., Fontanesi, E., Marasco, E., Mantovani, V., Pierini, M., Pini, E., Luiselli, D., Franceschi, C., Garagnani, P., 2012. Space/population and time/age in DNA methylation variability in humans: a study on IGF2/H19 locus in different Italian populations and in mono- and di-zygotic twins of different age. Aging (Albany N.Y.) 4, 509–520.
Rauser, C.L., Mueller, L.D., Rose, M.R., 2006. The evolution of late life. Ageing Res. Rev. 5, 14–32.
Romanoski, C.E., Glass, C.K., Stunnenberg, H.G., Wilson, L., Almouzni, G., 2015. Epigenomics: Roadmap for regulation. Nature 518 (7539), 314–316.
Santoro, A., Pini, E., Scurti, M., Palmas, G., Berendsen, A., Brzozowska, A., Pietruszka, B., Szczecinska, A., Cano, N., Meunier, N., de Groot, C.P., Feskens, E., Fairweather-Tait, S., Salvioli, S., Capri, M., Brigidi, P., Franceschi, C., NU-AGE Consortium, 2014, 2014. Combating inflammaging through a Mediterranean whole diet approach: the NU-AGE project's conceptual framework and design. Mech. Ageing Dev. 136–137 (March–April), 3–13.
Skytthe, A., Valensin, S., Jeune, B., Cevenini, E., Balard, F., Beekman, M., Bezrukov, V., Blanche, H., Bolund, L., Broczek, K., Carru, C., Christensen, K., Christiansen, L., Collerton, J.C., Cotichini, R., de Craen, A.J., Dato, S., Davies, K., De Benedictis, G., Deiana, L., Flachsbart, F., Gampe, J., Gilbault, C., Gonos, E.S., Haimes, E., Hervonen, A., Hurme, M.A., Janiszewska, D., Jylhä, M., Kirkwood, T.B., Kristensen, P., Laiho, P., Leon, A., Marchisio, A., Masciulli, R., Nebel, A., Passarino, G., Pelicci, G., Peltonen, L., Perola, M., Poulain, M., Rea, I.M., Remacle, J., Robine, J.M., Schreiber, S., Scurti, M., Sevini, F., Sikora, E., Skouteri, A., Slagboom, P.E., Spazzafumo, L., Stazi, M.A., Toccaceli, V., Toussaint, O., Törnwall, O., Vaupel, J.W., Voutetakis, K., Franceschi, C., C. GEHA consortium, 2011. Design, recruitment, logistics, and data management of the GEHA (Genetics of Healthy Ageing) project. Exp. Gerontol. 46, 934–945.